“Isolation, Biochemical Characterrization and Identification of Microorganisms from Spoilt Tomatoes Obtained from Local Markets of Dhaka City, Bangladesh”

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN MICROBIOLOGY

Submitted by:

Mahzabeen Chowdhury

Student ID: 14126005

September, 2018

Microbiology Program

Department of Mathematics and Natural Sciences

BRAC University

Dhaka, Bangladesh

Declaration

I hereby declare that the thesis project titled "**Isolation, Biochemical Characterization and Identification of Microorganisms from Spoilt Tomatoes Obtained from Local Markets of Dhaka City, Bangladesh**" has been submitted by me, Mahzabeen Chowdhury and has been carried out under the supervision of Nazneen Jahan, Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka.

It is further declared that this thesis has been composed solely by me and it has not been submitted, in whole or in part, in any previous institution for a degree or diploma. All explanations that have been adopted literally or analogously are marked as such.

_____

Candidate

(Mahzabeen Chowdhury)

_____

Certified by

(Nazneen Jahan)

Supervisor

Lecturer

Microbiology Program

Department of Mathematics and Natural Sciences

BRAC University, Dhaka.

DEDICATED

TO

MY

BELOVED MOTHER

Acknowledgement

First and foremost I would like to express my gratitude to the **Almighty Allah** because he has given me the opportunity and perseverance to finish this research.

My regards and gratitude go to Professor A **F M Yusuf Haider**, Chairperson of MNS Department, Professor **A.A. Ziauddin Ahmad (Late)**, Former Chairperson of MNS Department and Professor **Dr. Mahboob Hossain**, Coordinator of Microbiology Program of MNS Department of BRAC University for allowing me and encouraging me to complete my undergraduate thesis.

I would like to acknowledge my respected Supervisor **Nazneen Jahan**, Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University for her constant supervision, guidance and dedicated involvement to pursue new ideas and never-ending inspiration throughout the entire period of my research work. I would like to express my sincere gratitude me in my report writing. Without her invaluable assistance, this paper would have never been accomplished.

I would also like to thank the respective Lab officers **Shamim Akhter Chowdhury** and **Asma Binte Afzal**, Teaching assistants **Nahreen Mirza**, and **Salman Khan** for valuable suggestions and moral support during my work.

I am grateful to my thesis partner **Shorna Sheikh**, for providing her unfailing support throughout my work.

Finally, I would like to express profound gratitude to my parents and my friends for their continuous encouragement throughout the entire period of my research work.

Mahzabeen Chowdhury

September, 2018

Abstract

Tomato is a fruit which has high nutritional value along with uses in different dishes. This study aimed at isolating, identifying, and investigating enzyme activity and temperature tolerance of bacterial isolates collected from spoilt tomatoes of different markets of Dhaka city, Bangladesh. Six samples (1gm) of spoilt tomatoes collected with sterile knife and mixed with 5ml of saline and then cultured on various selective media. Identification of bacteria was done through conventional biochemical tests according to Bergey's Manual of Sysmetic Bacteriology. Temperature tolerance at temperatures (4^{o}C, 25^{o}C and 60^{o}C) of isolated bacteria were observed. Cellulose hydrolysis test was also performed using CMC Agar. A total of about 46 bacterial isolates were identified where *Vibrio* spp showed the highest prevalence 9 (19.56%), followed by *E.coli* 8 (17.39%), *Klebsiella* spp 7 (15.22%), *Salmonella* spp. 6 (13.04%), *Bacillus* spp. 6 (13.04%), *Shigella* spp 5 (10.87%), Staphylococcus spp. 3(6.52%) and *Enterobacter* spp. 2(4.65%). Temperature tolerance of bacterial isolates showed that all of the isolates were mesophiles and could not grow at temperatures of 4^{o}C and 60^{o}C. Cellulose hydrolysis test revealed that *Klebsiella* spp, *Shigella* spp, and *Salmonella* spp were able to hydrolyse cellulose. However, *E.coli*, *Vibrio* spp, and *Bacillus* spp, were not able to hydrolyse cellulose. These results indicate that spoilt tomatoes contain bacteria with ability to cause foodborne illness.

Table of contents:

List of tables:

List of figures:

List of Abbreviations:

MSA	Mannitol Salt Agar
MR	Methyl Red
VP	Voges-proskauer
TSI	Triple Sugar Iron
CMC	Carboxymethylcellulose Agar
MIU	Motility Indole Urease
spp.	Species
µl	Microliter

Chapter 1:

Introduction

1.1 Introduction

Tomato is an edible usually red color fruit of the plant Solanum lycopersicum known as tomato plant. The plant belongs to the nightshade family, *Solanaceae* [8, 9]. It is a common fruit in Bangladesh and is one of the most popular fresh market fruits due to various uses in salad, dishes, sauces, and drinks. Even though tomato is a fruit; it is generally regarded as culinary vegetables. Tomato is rich in vitamin C. It is usually grown as a winter vegetable in Bangladesh. The crop is sown mainly from October to November and becomes available for consumption from February to April (Amzad Hossain et al.,). During these times, tomatoes are available throughout the country. Dhaka being the capital of Bangladesh gets this fruit from all over the country. As a result, daily lots of tomatoes get lost due to various reasons and spoilage due to microbial spoilage is one of the leading cause. P K Sarma (2018) showed that in Bangladesh lots of tomatoes get lost after harvesting. Some reasons given were unfavorable weather condition, diseases and pests, damage during harvest, and damage during transport. If spoiled tomatoes are eaten, can cause health hazard leading to various diseases. For example, tomatoes had been linked to seven *Salmonella* outbreaks between 1990 and 2005 in North America according to the International food safety network.

To illustrate, bacteria associated with spoilage of carrots, potatoes, pepper, tomatoes, and cucumber of northern Nigeria markets were identified (F. M. Mahamud *et al*., 2012). While, spoilage related to a specific vegetable carrot has been identified from the Ose market of Onitsha, Nigeria (Onuorah Samuel et al., 2016).

1.2 Bacteria commonly found in spoiled tomatoes

Spoilage due to microorganisms depend on the process from post harvesting to the handling of the tomatoes. Types of bacteria may vary due to climate, environment, and surroundings. Generally some bacteria are found to be common in spoiled tomatoes from other studies and are responsible for foodborne illness. They are mentioned below:

- Escherichia coli-Only some strains of E.coli are harmful and causes diseases like intestinal infection. Symptoms due to intestinal infection are fever, diarrhea, and abdominal pain. Severe symptoms include bloody diarrhea, dehydration. Sources are contaminated water or food.

- Salmonella- Infection can occur through contaminated food or water. Salmonella causes salmonella infection known as salmonellosis with no symptoms or with symptoms like diarrhea, abdominal cramps, and fever.

- Bacillus cereus- Bacillus cereus is responsible for two types of foodborne illness as diarrhoeal syndromes and emetic (vomiting) (Schoeni and Wong 2005; Senesi and Ghelardi 2010). Contamination can occur through soil or through different kinds of food.

- Klebsiella-Infections in the urinary and lower biliary tract is caused by Klebsiella spp. (Lopes et al., 2005; Ryan, 2004). Immunocompromised person and hospitalized patients are at greater risks since Klebsiella is an opportunistic pathogen. (Podschun and Ullmann, 1998)

- Pseudomonas aeruginosa-This organism is found readily in soil, water and in nature. It is an opportunistic pathogen and causes infection in unhealthy individual. (Alice S. Prince 2012)

- Staphylococcus- The contamination food due to preformed S. aureus enterotoxins causes one of the most common foodborne illness known as Staphylococcal food-borne disease (SFD). Symptoms are vomiting, hypersalivation, nausea, and abdominal cramping with or without diarrhea. (Jhalka Kadariya et al., 2014)

1.3 Factors that affect food spoilage

Food spoilage is a natural process. All natural food decay with time. The variation is in only in duration of it. To prevent spoilage and maintain the quality of food, it is necessary to understand the causes of spoilage. Factors that can affect food spoilage include:

- Microorganisms
- Enzymes
- Light
- Insects, Rodents, Parasites and Other Creatures
- Physical Damage
- Temperature
- Time

Microorganisms

Food spoilage can happen due to microbial attack. Different types of microorganisms can cause the spoilage of food including fruits and vegetables. These microorganisms grow well in room temperatures as food stay in room temperatures. Spoilage microorganisms include bacteria, yeasts and molds. When spoilage occurs the food usually look and smell awful.

Enzymes

Enzymes are protein in nature and are organic catalyst produced by living cells. These are present naturally in food and are responsible for the ripening process in fruits and vegetables. The change in color, texture and flavor are done by enzymes. Enzymes of the food itself or by the enzymes produced by the microorganisms that contaminated the food may produce these changes. For instance, enzymes present within a raw fruit help it to ripen.

For spoilage, the microorganisms need to produce extracellular enzymes which can help in decay of the food. For example-cellulase, pectate lyase and polygalacturonase are some enzymes that help in spoilage. (M. Celia Marín-Rodríguez et al., 2002). For active penetration into fruits and vegetables, the microbes must be able to produce enzymes which dissolves the outer plant cell wall which predominantly consists of cellulose and pectin. Therefore, the presence of cellulase as one of these enzymes help in spoilage of the food.

Light

Light exposure could result in color and vitamin loss. Light also may be responsible for the oxidation of fats.

Insects, Rodents, Parasites and Other Creatures

These organisms can cause a lot of damage. Along with eating the fruit, they can also pass microorganisms through their hairs and droppings too. The affected parts are then become more susceptible to diseases.

Physical Damage

Physical damage like bruises or cracks due to falling, crushing, pressure which causes the peel of the fruits to damage increases the chance of spoilage. These provide places for microorganisms, light, and creatures to enter.

Temperature

Temperature affects the deterioration time and fruits degenerate faster at higher temperatures. Very high temperatures kill the microbes and the enzymes are denatured. Also, in low temperatures the microbial growth is slow and enzyme activity too. Usually at room temperature, microorganisms both spoilage and pathogenic grow rapidly.

Time

Time is required for microorganism to grow and multiply. Other reactions like enzyme action also need time to develop.

1.4 Prevention against spoilage

Lots of tomatoes are lost due to spoilage. As tomatoes are usually carried from production areas to consumption areas in locally woven baskets and sacks under conditions which encourage the growth of microorganisms. Therefore, prevention measures against it are necessary. For example, good agricultural practices (GAPs) and good manufacturing practices (GMPs) during cultivation, harvest, storage, transport, and marketing should be practiced. At home, fruits should be stored in refrigerators as cold temperature slows down enzyme activity and prevent growth of microorganisms. Thorough washing of fruits with clean water is also recommened to remove any dirt or insecticide residues. They can be preserved by heating that deactivates the enzymes and kill the microbes. The fruits can also be preserved by drying or souring them.

1.5 Literature Review

Mahamud et al., (2013) identified bacteria responsible for spoilage of some vegetables like carrot, tomatoes, cucumber, pepper, potatoes from Kaduna central market and Kawo market of Northern Nigeria. The presence of both Gram positive and Gram negatives were found. For examples- Staphylococcus, Streptococcus strains with Escherichia coli, Citrobacter and Klebsiella were identified from Kaduna central market vegetables where the most abundant one with 80% relative occurrence was Staphylococcus and the least is Streptococcus with 10% relative abundance. Also, Klebsiella, Escherichia coli, Citrobacter were found among Gram negative with 30% abundance each. Enterobacter with the least percentage was present too. Similar result was found from the other market with difference of absence of E.coli and the presence of *Edwardsiella spp. S. aureus* and *Klebsiella* were found with highest percentage in Kawo market.

Bashir Omolaran Bello et al., (2016) investigated the presence of bacteria and fungi on both healthy and decayed tomatoes to make comparison from Sabo market and Oja-Oba market in Nigeria. Two types of bacteria *Staphylococcus* and *Bacillus* species and two fungi *Aspergillus flavus, Rhizopus stolonifer* were isolated from both healthy and decayed tomatoes. However, the prevalence of microbes were more for Sabo market than Oja-Oba market. The results of pathogenity tests also showed fruit decay was caused by both bacteria and fungi observed.

J. Raja Brindha et al., (2011) worked on enzymatic activity of fungal organisms causing spoilage in tomato. Degradation of cellulose and pectin which are the polymeric coumpounds present in tomato help in spoilage of tomato. Therefore, the study linked the relationship between fungi and the extracellular enzymes produced by these fungi related to spoilage of the fruit. Three different isolates *Aspergillus, Penicillum* and *Trichoderma* from the spoilt tomatoes were screened for enzymatic activity. *Aspergillus* sp produced amylase of maximum 48U/ml at 72hrs of incubation period in submerged fermentation. While *Trichoderma* formed cellulose of maximum 48U/ml at 72 hrs of incubation period. These enzymes have commercial values in various industries.

1.6 Aims and objectives

This analysis was focused on isolation, biochemical characterization and identification of microorganisms from spoilt tomatoes.

The objective of the study is

- to determine the microorganisms responsible for spoilage of tomatoes
- to determine the frequency of occurrence of isolated bacteria and
- biochemical characterization of spoilage causing bacterial isolates
- determine the presence of spoilage related enzymes in isolated microbes
- sustaining ability of isolated microbes in different temperatures

Chapter 2: Materials & Methods

Materials and Methods:

2.1 Study area and duration:

The laboratory processing, analysis of data and the overall experimental work were done in Microbiology Research Laboratory of the Department of Mathematics of Mathematics and Natural sciences of BRAC University. The research was conducted during the period of January-September, 2018.

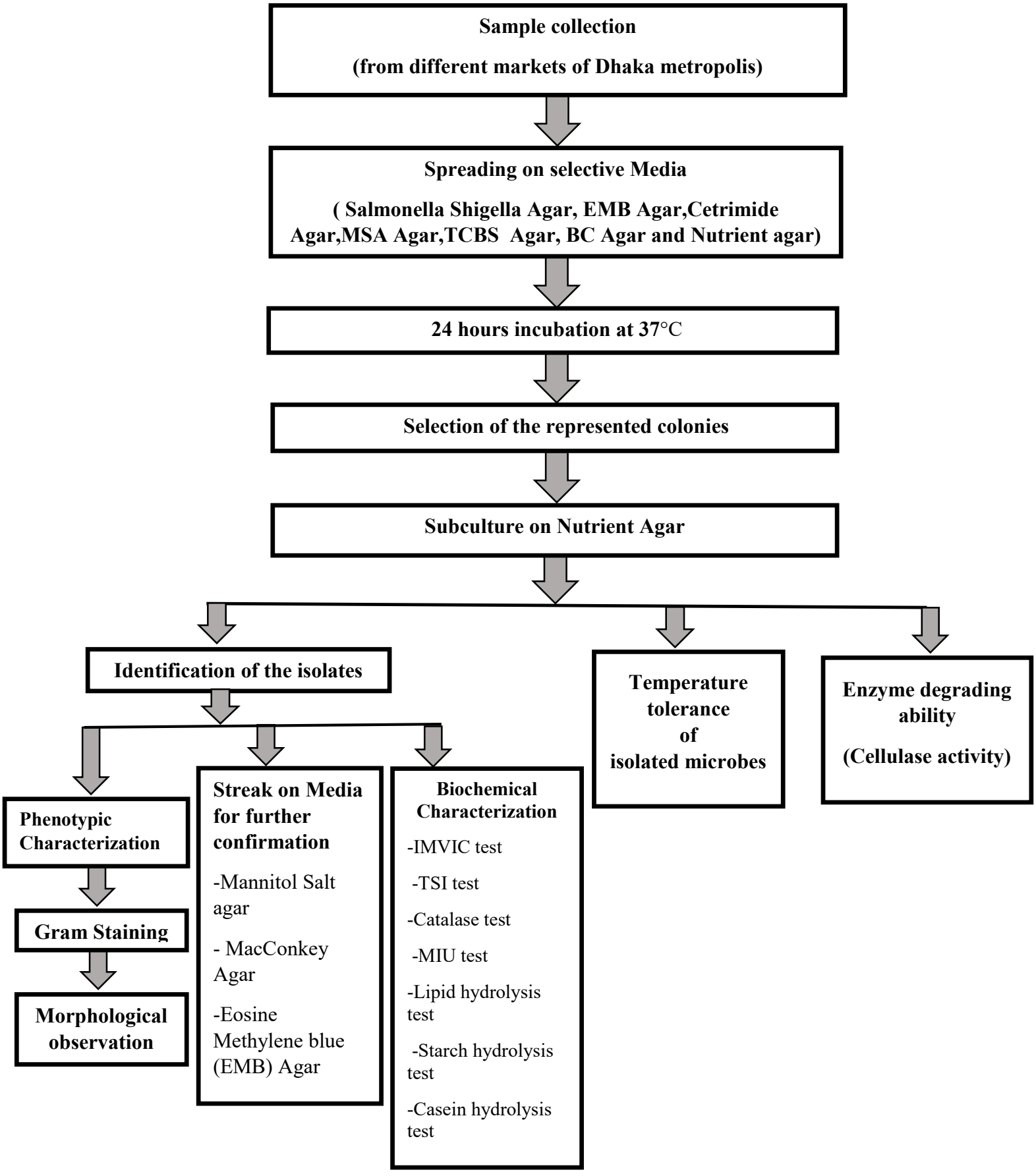
2.2 Sample size:

Spoiled tomato samples from six different bazars of Dhaka metropolis were collected.

2.3 Sample collection and processing:

A total of six samples from different markets of Dhaka metropolis were brought using sterile plastic bags for each. Then, they were immediately transported to the Microbiology Research Laboratory of BRAC University. Next, 1gm of spoiled area of tomato was cut off using sterile knife and mixed with 5ml of saline. The prepared sample was further used for analysis.

2.4 Experimental design:



2.5 Isolation, purification and storage of sample:

Sources of 6 samples collected and their respective collection date, time, and number of isolates are mentioned below:

Table 2.1: Sample Collection: Source, Date, Number of the isolates found and their given name in the study

Sample No.	Source	Date	Number of the isolates found	Isolates ID
1	Uttara-13 Bazar	21/01/18	9	T1a-T1i
2	Karon Bazar	28/01/18	6	T2a-T2f
3	Adabor-12 Bazar	11/02/18	7	T3a-T3g
4	Tongi Bazar	25/02/18	6	T4a-T4f
5	Town Hall	04/03/18	8	T5a-T5h
6	Banani Bazar	11/03/18	7	T6a-T6g

After the samples were collected, they were spread on different selective agar to isolate the microorganisms. Each prepared sample was spread on Nutrient Agar from dilutions 10^{-1} to 10^{-5}. Also, prepared samples were spread directly on different selective media plates (Mannitol Salt Agar, Eosine Methylene Blue Agar, Bacillus Cereus Agar, Salmonella Shigella Agar, TCBS agar, Cetrimide Agar). Then all the plates were incubated for 24 hours at 37^{o}C. Further the isolates from the nutrient agar and selective media plates were streaked on nutrient agar plates to get pure cultures for storage.

Long term preservation:

T_1N_1 stock media was prepared in a sterile vial. Bacteria was taken from culture plate with sterile inoculating needle and stabbed into the 3ml T_1N_1 media. Then, it was incubated for 24 hours at 37ºC. After that 300 µl of sterile glycerol was added to the inoculated T_1N_1 media and the vial was stored at room temperature.

2.6 Biochemical identification:

Methods from Bergey's Manual of Systematic bacteriology was used for the biochemical identification of the isolates.

2.6.1 Indole test

Indole production test was done to determine the ability of microorganisms to degrade the amino acidtryptophan by the enzyme tryptophanase.

- For indole test each indole broth containing 6ml of peptone, sodium chloride was taken.
- Using sterile technique, small amount of the experimental bacteria from fresh culture was inoculated into the tubes by means of loop inoculation method with an inoculating loop
- The tubes were then incubated for 24 hours at37ºC.
- In order to detect the indole production, 10 drops of Kovacs reagent was added to all the tubes.
- If red reagent layer develops then it indicates indole positive and absence of red color indicates that the substrate tryptophan was not hydrolyzed and it indicates indole negative reaction. (Cappuccino &Sherman, 2005)

2.6.2 Methyl Red test

Methyl red test was done to determine the ability of the bacteria to oxidize glucose with the production and stabilization of high concentration of acid end products.

- For methyl red test each MR broth containing 5 ml of dipeptone, dextrose and potassium phosphate was taken.

- Using sterile technique, each tube was inoculated by fresh culture of experimental bacteria by means of loop inoculation method.
- The tubes were then incubated for 48 hours at37ºC.
- After 48 hours, 5 drops of methyl red indicator was added to each tube and the colour of the tubes was observed.
- If red colour develops then it indicates that the organism was capable of fermenting glucose with the production of high concentration of acid.
- If orange or yellow colour develops then it indicates methyl red negative result (Cappuccino &Sherman, 2005).

2.6.3 Voges-Proskauer (VP) test

The Voges-Proskauer (VP) test was done to determine if an organism produces acetyl methyl carbinol from glucose fermentation.

- For Voges-Proskauer test each VP broth containing dipeptone, dextrose and potassium phosphate was taken.
- Using sterile technique, each tube was inoculated by fresh culture of experimental bacteria by means of loop inoculation method.
- The tubes were then incubated for 48 hours at37ºC.
- After 48 hours, 10 drops of Barritt's reagent A was added to each tube and the tubes were shaken. Then immediately 10 drops of Barritt's reagent B was added and the tubes were shaken.
- The colour was observed after 15-30 minutes of the reagent addition.
- If red colour developed then it indicates that the organism was capable of fermenting glucose with ultimate production of acetyl methyl carbinol and it indicates positive result.
- If no colour developed then it indicates voges- proskauer negative result. (Cappuccino &Sherman, 2005)

2.6.4 Citrate utilization test

Citrate utilization test was done to differentiate among enteric organisms on the basis of their ability to ferment citrate as a sole source of carbon by the enzyme citrase.

- For citrate utilization test each vial containing 2.5 ml of Simmons citrate agar was taken.
- Using sterile technique, small amount of the experimental bacteria from 24-hours fresh culture was inoculated into the vials by means of a streak inoculation method with an inoculating loop.
- The vials were then incubated at 37ºC for 24-48 hours.
- After 48 hours incubation, if the Prussian blue colour developed then it indicates the citrate positive result which means the organism was capable of fermenting citrate as a sole source of carbon.
- If there was no colour change then it indicates citrate negative result.

2.6.5 Triple sugar-iron (TSI) agar test

Triple sugar iron agar test was done to differentiate between Gram negative enteric bacilli based on their ability to ferment carbohydrate and reduce hydrogen sulfide.

- For TSI test each tube containing TSI agar was taken.
- Using sterile technique, small amount of the experimental bacteria from fresh culture was inoculated into the tubes by means of stab inoculation method with an inoculating needle.
- The tubes were then incubated at 37˚C for 24-48 hours.
- After 24-48 hours the color of both the butt and slant of agar slant cultures were observed.

2.6.6 Catalase test

The differentiation of bacteria that produce the enzyme catalase from non-catalase producers is achieved using this test. Catalase acts as a catalyst in the breaking down of hydrogen peroxide to Oxygen and water, two to three ml of 3% hydrogen peroxide solution was poured into a test tube. A 24 hour culture of the test organism from the nutrient agar was emulsified in the hydrogen

peroxide solution. The release of bubbles immediately indicated a positive test while it was negative when no bubble was formed.

2.6.7 MIU (Motility-indole -urease) test

MIU test was done for determining the motility of bacteria, indole production and urea degradation by means of the enzyme urease.

- Using sterile technique, small amount of the experimental bacteria from fresh culture was inoculated into the tubesby means of stab inoculation method with an inoculating needle
- The tubes were then incubated for 24 hours at 37ºC.
- The growth of the organism would spread throughout the test tube from downward to the upward of the test tube, if the organism is motile.
- The colour of the media will turn to deep pink if the organism is positive for urease test. If yellow colour develops then it indicates urease negative result.
- To confirm the indole test, five drops of Kovac's reagent was added following overnight incubation. Then the colour of the media was examined and the results were recorded.
- Formation of a rose red ring at the top indicates a positive result. A negative result can have a yellow or brown layer (Cappuccino &Sherman, 2005).

2.6.8 Starch Hydrolysis test

Starch hydrolysis test was done to observe if the microbes can use starch, a complex carbohydrate made from glucose, as a source of carbon and energy for growth. Use of starch is accomplished by an enzyme called alpha-amylase.

- Soluble starch media was dissolved in a small amount of water and was heated slowly with constant stirring. Then all the ingredients were added to it and was transferred into a conical flask and sterilized by autoclaving at 121.5°C.
- The sterilized agar medium was poured into the sterilized Petri plates and was allowed to solidify.

- Each plate was inoculated at the center with the bacterial inoculum.
- Plates were incubated at 37°C for 24–48 hrs.
- To test the hydrolysis of starch, each plate was flooded with iodine.

An appearance of clear zone around the growth is considered as positive result.(Cappuccino

2.6.9 Casein Hydrolysis test

This test was done to determine the ability of microorganisms to excrete hydrolytic extracellular enzymes capable of degrading the protein casein.

- Using sterile technique, skim milk agar plates were inoculated with the test organism by using a sterile inoculating loop.
- Then the plates were incubated for 24 hours at 37°C.
- If the organisms secrete proteases, it will exhibit a zone of proteolysis which is demonstrated by a clear area surrounding the bacterial growth. It represents a positive result. In the absence of protease activity, the medium surrounding growth of the organism remains opaque which is a negative result.

2.6.10 Lipid Hyrolysis test

This media tests for the ability of an organism to break down and use a vegetable lipid (tributyrin) present in the agar plates. If an organism is able to secrete lipase the lipid can be hydrolyzed. The media usually contains spirit blue or methylene blue as an indicator. Use of the lipid can be observed as a zone of clearing around areas of growth. The zone has to be transparent for the test to be considered positive; color changes are not considered to be positive.

Chapter 3: Result

3.1 Bacterial isolation and identification:

3.1.1 Cultural and morphological characteristics of the bacterial isolates:

In table 3.1 the color, shape of the colonies on various selective and differential media and the morphology of the bacterial colonies on nutrient agar are explained.

Table 3.1 Cultural and morphological characteristics of the bacterial isolates from spoilt tomatoes on various Selective and Differential media and Nutrient Agar

	Growth on Selective, and Differential Media						Colony morphology on Nutrient Agar					Suspect
Isolates ID	**Cetrimide**	**Mannitol Salt Agar (MSA**	**Eosine Methylene Blue Agar (EMB)**	**Bacillus Cereus Agar (BC**	**Salmonella Shigella Agar (SSA)**	**TCBS agar**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	
T1a	-	-	Metallic Green sheen colonies	-	-	-	Medium	Creamy	Circular	Entire	Raised	***E.coli***
T1b	-	-	-	Blue colored colonie	-	-	Large	White	Circular	Entire	Convex	***Bacillus* spp**
T1c	-	-	-	-	-	Yellow coloured	Small	White	Circular	Entire	Flat	***Vibrio* spp**
T1d	-	-	-	-	-	Green coloured	Small	White	Circular	Entire	Flat	***Vibrio* spp**
T1e	-	-	-	-	Black centered colourl	-	Medium	White	Circular	Entire	Convex	***Samonella* spp**
T1i	-	-	Metallic Green sheen colonies	-	-	-	Medium	Creamy	Circular	Entire	Raised	***E.coli***

Table 3.1 Cultural and morphological characteristics of the bacterial isolates from spoilt tomatoes on various Selective and Differential media and Nutrient Agar

	Growth on Selective, and Differential Media						Colony morphology on Nutrient Agar					Suspected Organism
Isolates ID	Cetrimide	Mannitol Salt Agar (MSA)	Eosine Methylene Blue Agar (EMB)	Bacillus Cereus Agar (BC Agar)	Salmonella Shigella Agar (SSA)	TCBS agar	Size	Color	Form	Margin	Elevation	
T1f	-	-	-	-	Colorless colonies	-	Small	Colorless	Circular	Entire	Convex	***Shigella spp.***
T1g	-	-	Pink colored mucoid colonies	-	-	-	Large	Creamy	Circular	Entire	Flat	***Klebsiella spp.***
T1h	-	-	Pink colored colonies	-	-	-	Small	Creamy	Circular	Entire	Convex	***Enterobact-er* spp.**
T2e	-	-	-	-	Colorless colonies	-	Small	Colorless	Circular	Entire	Convex	**Shigella spp**
T2d	-	-	-	-	Black centered colorless colonies	-	Medium	White	Circular	Entire	Convex	**Samonella spp**

Table 3.1 Cultural and morphological characteristics of the bacterial isolates from spoilt tomatoes on various Selective and Differential media and Nutrient Agar

	Growth on Selective, and Differential Media						Colony morphology on Nutrient Agar					Suspected Organism
Isolates ID	**Cetrimide**	**Mannitol Salt Agar (MSA)**	**Eosine Methylene Blue Agar (EMB)**	**Bacillus Cereus Agar (BC Agar)**	**Salmonella Shigella Agar**	**TCBS agar**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	
T2c	-	-	-	-	-	Yellow colored colonies	Small	White	Circular	Entire	Flat	***Vibrio spp.***
T2f	-	-	Pink colored mucoid colonies	-	-	-	Large	Creamy	Circular	Entire	Flat	***Klebsiella spp.***
T2b	-	-	-	-	-	Blue colored colonies	Large	White	Circular	Entire	Convex	***Bacillus spp.***
T2a	-	-	Metallic Green sheen colonies	-	-	-	Medium	Creamy	Circular	Entire	Raised	***E.coli***
T2g	-	Small, yellow colored colonie	-	-	-	-	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**
T3d	-	-	Pink, colored colonies	-	-	-	Small	Creamy	Circular	Entire	Convex	***Enterobacter* spp.**
	-	Small, yellow colored	-	-	-	-	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.1 Cultural and morphological characteristics of the bacterial isolates from spoilt tomatoes on various Selective and Differential media and Nutrient Agar

	Growth on Selective, and Differential Media						Colony morphology on Nutrient Agar					Suspected Organism
Isolates ID	**Cetrimide**	**Mannitol Salt Agar (MSA)**	**Eosine Methylene Blue Agar (EMB)**	**Bacillus Cereus Agar (BC Agar)**	**Salmonella Shigella Agar (SSA)**	**TCBS agar**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	
T3c	-	-	-	-	-	Yellow colored colonies	Small	White	Circular	Entire	Flat	***Vibrio spp.***
T3g	-	-	Pink colored mucoid colonies	-	-	-	Large	Creamy	Circular	Entire	Flat	***Klebsiella spp.***
T3b	-	-	-	-	-	Blue colored colonies	Large	White	Circular	Entire	Convex	***Bacillus spp.***
T3a	-	-	Metallic Green sheen colonies	-	-	-	Medium	Creamy	Circular	Entire	Raised	***E.coli***
T3e	-	-	-	-	Black centered colorless colonies	-	Medium	White	Circular	Entire	Convex	***Salmonella* spp.**

Table 3.1 Cultural and morphological characteristics of the bacterial isolates from spoilt tomatoes on various Selective and Differential media and Nutrient Agar

	Growth on Selective, and Differential Media							Colony morphology on Nutrient Agar					Suspected Organism
Isolates ID	**Cetrimide**		**Mannitol Salt Agar (MS**	**Eosine Methylene Blue Agar (EMB)**	**Bacillus Cereus Agar (BC Agar)**	**Salmonella Shigella Agar (SSA)**	**TCBS agar**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	
T3f	-		-	-	-	Colorless colonies	-	Small	Colorless	Circular	Entire	Convex	***Shigella spp.***
T4f	-		-	Pink colored mucoid colonies	-	-	-	Large	Creamy	Circular	Entire	Flat	***Klebsiella spp.***
T4a	-		-	Metallic Green sheen colonies	-	-	-	Medium	Creamy	Circular	Entire	Raised	***E.coli***
T4c	-		-	-	-	-	Green colored colonies	Small	White	Circular	Entire	Flat	***Vibrio* spp**
T4e	-		-	-	-	Black centered colorless colonies	-	Medium	White	Circular	Entire	Convex	**Samonella spp**

Table 3.1 Cultural and morphological characteristics of the bacterial isolates from spoilt tomatoes on various Selective and Differential media and Nutrient Agar

	Growth on Selective, and Differential Media						Colony morphology on Nutrient Agar					Suspected Organism
Isolates ID	**Cetrimide**	**Mannitol Salt Agar (MSA)**	**Eosine Methylene Blue Agar (EMB)**	**Bacillus Cereus Agar (BC Agar)**	**Salmonella Shigella Agar (SSA)**	**TCBS agar**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	
T4b	-	-	-	Blue colored colonies	-	-	Large	White	Circular	Entire	Convex	***Bacillus spp***
T4d	-	-	-	-	-	Yellow coloured coonies	Small	White	Circular	Entire	Flat	***Vibrio* spp**
T5a	-	-	Metallic Green sheen colonies	-	-	-	Medium	Creamy	Circular	Entire	Raised	***E.coli***
T5e	-	-	-	-	Black centered colourless colonies	-	Medium	White	Circular	Entire	Convex	***Samonella* spp**
T5g	-	-	-	-	Colorless colonies	-	Small	Colorless	Circular	Entire	Convex	***Shigella* spp.**

Table 3.1 Cultural and morphological characteristics of the bacterial isolates from spoilt tomatoes on various Selective and Differential media and Nutrient Agar

	Growth on Selective, and Differential Media						Colony morphology on Nutrient Agar					Suspected Organism
Isolates ID	**Cetrimide**	**Mannitol Salt Agar (MSA)**	**Eosine Methylene Blue Agar (EMB)**	**Bacillus Cereus Agar (BC Agar)**	**Salmonella Shigella Agar (SSA)**	**TCBS agar**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	
T5i	-	-	Metallic Green sheen coloniess	-	-	-	Medium	Creamy	Circular	Entire	Raised	***E.coli***
T5b	-	-	-	Blue colored colonies	-	-	Large	White	Circular	Entire	Convex	***Bacillus spp***
T5c	-	-	-	-	-	Yellow coloured coonies	Small	White	Circular	Entire	Flat	**Vibrio spp**
T5d	-	-	-	-	-	Green coloured	Small	White	Circular	Entire	Flat	**Vibrio spp**
T5h	-	-	Pink colored mucoid colonies	-	-	-	Large	Creamy	Circular	Entire	Flat	***Klebsiella* spp.**
T5j	-	Small, yellow colored colonies	-	-	-	-	Small	Yellow	Circular	Entire	Convex	***Staphylococcus* spp.**

Table 3.1 Cultural and morphological characteristics of the bacterial isolates from spoilt tomatoes on various Selective and Differential media and Nutrient Agar

	Growth on Selective, and Differential Media						Colony morphology on Nutrient Agar					Suspected
Isolates ID	**Cetrimide**	**Mannitol Salt Agar (MSA)**	**Eosine Methylene Blue Agar (EMB)**	**Bacillus Cereus Agar (BC Agar**	**Salmonella Shigella Agar (SSA)**	**TCBS agar**	**Size**	**Color**	**Form**	**Margin**	**Elevation**	
T6a	-	-	Metallic Green sheen	-	-	-	Medium	Creamy	Circular	Entire	Raised	***E.coli***
T6b	-	-	-	Blue colored	-	-	Large	White	Circular	Entire	Convex	***Bacillus spp***
T6c	-	-	-	-	-	Yellow coloured coonies	Small	White	Circular	Entire	Flat	**Vibrio spp**
T6e	-	-	-	-	Colorless colonies	-	Small	Colorless	Circular	Entire	Convex	***Shigella* spp.**
T6d	-	-	-	-	Black centered colourless	-	Medium	White	Circular	Entire	Convex	**Samonella spp**
T6f	-	-	Pink colored mucoid colonies	-	-	-	Large	Creamy	Circular	Entire	Flat	***Klebsiella* spp.**
T6g	-	-	Pink colored mucoid colonies	-	-	-	Large	Creamy	Circular	Entire	Flat	***Klebsiella* Spp.**

3.1.2 Biochemical characteristics of the bacterial isolates

Table 3.2: Biochemical characteristics of the bacteria isolated from spoilt tomatoes

Isolates Id	Gram Staining		Indole Test	Methyl red Test	VP Test	Citrate Test	Triple Sugar Iron Test						Carbohydrate fermentation		MIU Test			Catalase Test	Starch Hydrolysis	Casein Hydrolysis	Lipid Hydrolysis	Suspected Organism
	Gram reaction	Shape					Slant/Butt	Glucose	Lactose	Sucrose	H2S	Gas	Maltose	Gas	Motility	Indole	Urease					
T1a	-	rod	+	+	-	-	Y/Y	+	+	+	-	+	+	+	+	+	-	+	-	-	-	E.coli
T1b	+	rod	-	-	-	-	R/Y	+	-	-	-	-	+	-	-	-	-	+	-	-	+	Bacillus spp.
T1c	-	rod	-	-	-	-	Y/Y	+	+	+	-	-	+	+	+	-	-	+	-	+	+	Vibrio spp.
T1d	-	rod	+	-	-	+	R/R	-	-	-	-	-	+	-	+	+	-	+	+	+	+	Vibrio spp.
T1e	-	rod	-	+	-	+	Y/B	+	+	+	+	+	+	-	+	-	-	+	-	-	-	Salmonella
T1i	-	rod	+	+	-	-	Y/Y	+	+	+	-	+	+	+	+	+	-	+	-	-	-	E.coli
T1f	-	rod	-	+	-	-	R/Y	+	-	-	-	+	+	+	-	-	-	+	-	+	-	Shigella
T1g	-	rod	-	-	+	+	Y/Y	+	+	+	-	+	+	+	-	-	+	+	-	+	-	Klebsiella spp.

Table 3.2: Biochemical characteristics of the bacteria isolated from spoilt tomatoes

Isolates Id	Gram Staining		Indole Test	Methyl red Test	VP Test	Citrate Test	Triple Sugar Iron Test						Carbohydrate fermentation		MIU Test			Catalase Test	Starch Hydrolysis	Casein Hydrolysis	Lipid Hydrolysis	Suspected Organism
	Gram reaction	Shape					Slant/Butt	Glucose	Lactose	Sucrose	H2S	Gas	Maltose	Gas	Motility	Indole	Urease					
T1h	-	rod	-	-	+	+	Y/Y	+	+	+	-	+	+	+	+	-	-	+	-	-	-	Enterobacter spp.
T2e	-	rod	-	+	-	-	R/Y	+	-	-	-	+	+	+	-	-	-	+	-	+	-	Shigella spp.
T2d	-	rod	-	+	-	+	Y/B	+	+	+	+	+	+	-	+	-	-	+	-	-	-	Salmonella spp.
T2c	-	rod	-	-	-	-	Y/Y	+	+	+	-	-	+	+	+	-	-	+	-	+	+	Vibrio spp.
T2f	-	rod	-	-	+	+	Y/Y	+	+	+	-	+	+	+	-	-	+	+	-	+	-	Klebsiella spp.
T2b	+	rod	-	-	-	-	R/Y	+	-	-	-	-	+	-	-	-	-	+	-	-	+	Bacillus spp.
T2a	-	rod	+	+	-	-	Y/Y	+	+	+	-	+	+	+	+	+	-	+	-	-	-	E.coli
T2g	+	cocci	-	+	-	-	Y/Y	+	+	+	-	-	+	-	-	-	+	+	-	+	+	Staphylococcus spp.

Table 3.2: Biochemical characteristics of the bacteria isolated from spoilt tomatoes

Isolates Id	Gram Staining		Indole Test	Methyl red Test	VP Test	Citrate Test	Triple Sugar Iron Test						Carbohydrate fermentation		MIU Test			Catalase Test	Starch Hydrolysis	Casein Hydrolysis	Lipid Hydrolysis	Suspected Organism
	Gram reaction	Shape					Slant/Butt	Glucose	Lactose	Sucrose	H2S	Gas	Maltose	Gas	Motility	Indole	Urease					
T3d	-	rod	-	-	+	+	Y/Y	+	+	+	-	+	+	+	+	-	-	+	-	-	-	Enterobacter spp
T3h	+	cocci	-	+	-	-	Y/Y	+	+	+	-	-	+	-	-	-	+	+	-	+	+	Staphylococcus spp
T3c	-	rod	-	-	-	-	Y/Y	+	+	+	-	-	+	+	+	-	-	+	-	+	-	Vibrio spp.
T3g	-	rod	-	-	+	+	Y/Y	+	+	+	-	+	+	+	-	-	+	+	-	+	-	Klebsiella spp
T3b	+	rod	-	-	-	-	R/Y	+	-	-	-	-	+	-	-	-	-	+	-	-	+	Bacillus spp
T3a	-	rod	+	+	-	-	Y/Y	+	+	+	-	+	+	+	+	+	-	+	-	-	-	E.coli
T3e	-	rod	-	+	-	+	Y/B	+	+	+	+	+	+	-	+	-	-	+	-	-	-	Salmonella spp
T3f	-	rod	-	+	-	-	R/Y	+	-	-	-	+	+	+	-	-	-	+	-	+	-	Shigella spp

Table 3.2: Biochemical characteristics of the bacteria isolated from spoilt tomatoes

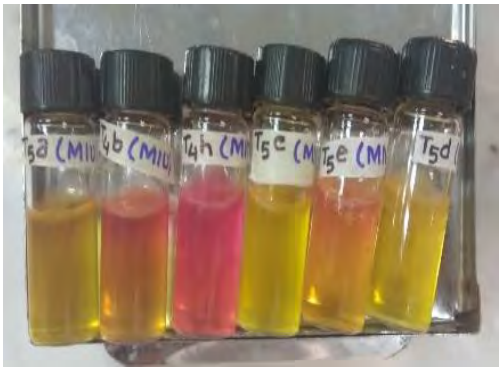
Isolates Id	Gram Staining		Indole Test	Methyl red Test	VP Test	Citrate Test	Triple Sugar Iron Test						Carbohydrate fermentation		MIU Test			Catalase Test	Starch Hydrolysis	Casein Hydrolysis	Lipid Hydrolysis	Suspected Organism
	Gram reaction	Shape					Slant/Butt	Glucose	Lactose	Sucrose	H2S	Gas	Maltose	Gas	Motility	Indole	Urease					
T4f	-	rod	-	-	+	+	Y/Y	+	+	+	-	+	+	+	-	-	+	+	-	+	-	Klebsiella spp.
T4a	-	rod	+	+	-	-	Y/Y	+	+	+	-	+	+	+	+	+	-	+	-	-	-	E.coli
T4c	-	rod	-	-	-	-	Y/Y	+	+	+	-	-	+	+	+	-	-	+	-	+	+	Vibrio spp.
T4e	-	rod	-	+	-	+	Y/B	+	+	+	+	+	+	-	+	-	-	+	-	-	-	Salmonella spp.
T4b	+	rod	-	-	-	-	R/Y	+	-	-	-	-	+	-	-	-	-	+	-	-	+	Bacillus spp.
T4d	-	rod	+	-	-	+	R/R	-	-	-	-	-	+	-	+	+	-	+	+	+	+	Vibrio spp.
T5a	-	rod	+	+	-	-	Y/Y	+	+	+	-	+	+	-	+	+	-	+	-	-	-	E.coli
T5e	-	rod	-	+	-	+	Y/B	+	+	+	+	+	+	-	+	-	-	+	-	-	-	Salmonella spp.

Table 3.2: Biochemical characteristics of the bacteria isolated from spoilt tomatoes

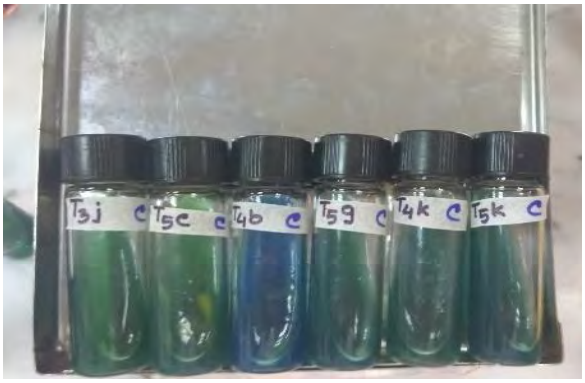
Isolates Id	Gram Staining		Indole Test	Methyl red Test	VP Test	Citrate Test	Triple Sugar Iron Test						Carbohydrate fermentation		MIU Test			Catalase Test	Starch Hydrolysis	Casein Hydrolysis	Lipid Hydrolysis	Suspected Organism
	Gram reaction	Shape					Slant/Butt	Glucose	Lactose	Sucrose	H2S	Gas	Maltose	Gas	Motility	Indole	Urease					
T5g	-	rod	-	+	-	-	R/Y	+	-	-	-	+	+	+	-	-	-	+	-	+	-	Shigella spp.
T5i	-	rod	+	+	-	-	Y/Y	+	+	+	-	+	+	+	+	+	-	+	-	-	-	E.coli
T5b	+	rod	-	-	-	-	R/Y	+	-	-	-	-	+	-	-	-	-	+	-	-	+	Bacillus spp.
T5c	-	rod	-	-	-	-	Y/Y	+	+	+	-	-	+	+	+	-	-	+	-	+	+	Vibrio spp.
T5d	-	rod	+	-	-	+	R/R	-	-	-	-	-	+	-	+	+	-	+	+	+	+	Vibrio spp.
T5h	-	rod	-	-	+	+	Y/Y	+	+	+	-	+	+	+	-	-	+	+	-	+	-	Klebsiella spp.
T5j	+	cocci	-	+	-	-	Y/Y	+	+	+	-	-	+	-	-	-	+	+	-	+	+	Staphylococcus spp.
T6a	-	rod	+	+	-	-	Y/Y	+	+	+	-	+	+	+	+	+	-	+	-	-	-	E.coli

Table 3.2: Biochemical characteristics of the bacteria isolated from spoilt tomatoes

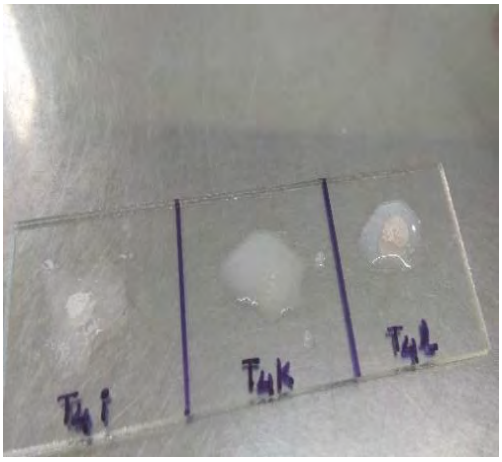
Isolates Id	Gram Staining		Indole Test	Methyl red Test	VP Test	Citrate Test	Triple Sugar Iron Test						Carbohydrate fermentation		MIU Test			Catalase Test	Starch Hydrolysis	Casein Hydrolysis	Lipid Hydrolysis	Suspected Organism
	Gram reaction	Shape					Slant/Butt	Glucose	Lactose	Sucrose	H2S	Gas	Maltose	Gas	Motility	Indole	Urease					
T6b	+	rod	-	-	-	-	R/Y	+	-	-	-	-	+	-	-	-	-	+	-	-	+	Bacillus spp.
T6c	-	rod	-	-	-	-	Y/Y	+	+	+	-	-	+	+	+	-	-	+	-	+	+	Vibrio spp.
T6e	-	rod	-	+	-	-	R/Y	+	-	-	-	+	+	+	-	-	-	+	-	+	-	Shigella spp.
T6d	-	rod	-	+	-	+	Y/B	+	+	+	+	+	+	-	+	-	-	+	-	-	-	Salmonella spp.
T6f	-	rod	-	-	+	+	Y/Y	+	+	+	-	+	+	+	-	-	+	+	-	+	-	Klebsiella spp.
T6g	-	rod	-	-	+	+	Y/Y	+	+	+	-	+	+	+	-	-	+	+	-	+	-	Klebsiella spp.



MIU Test



Citrate Test

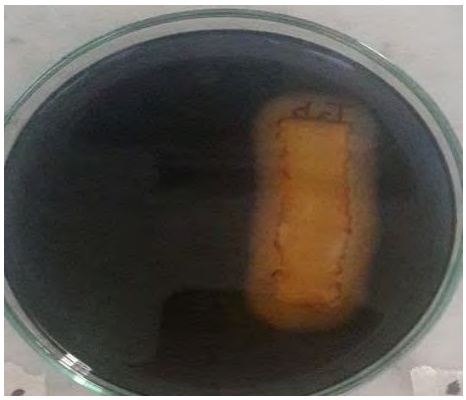


Catalase Test

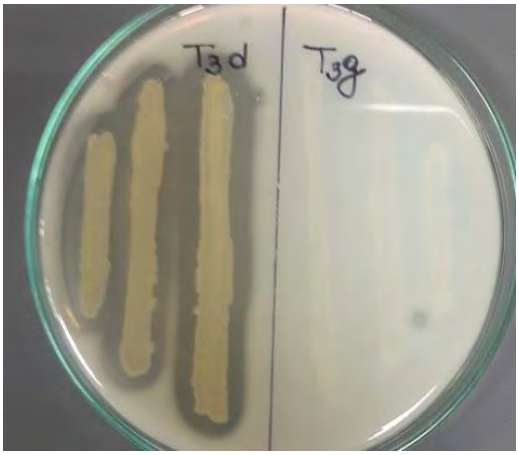
Voges-Proskauer Test

Figure 3.1 Biochemical test results of bacterial isolates

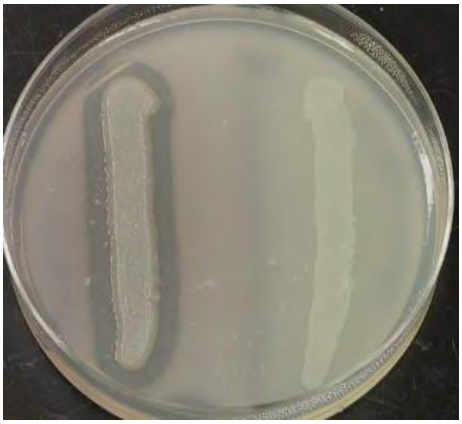
TSI Test



Starch Hydrolysis Test



Casein Hydrolysis test



Lipid Hydrolysis Test

Figure 3.1 Biochemical test results of bacterial isolates

At the end of studying the cultural and morphological characteristics of bacterial isolates and completing biochemical test, 46 isolates have been identified from the six tomato samples collected from different markets of Dhaka metropolis. The isolates were identified as *E.coli*, *Bacillus* spp, *Vibrio* spp, *Salmonella* spp, *Shigella* spp, *Enterobacter* spp, and *Klebsiella* spp. The total number of identified isolates along with the percentage of the isolates obtained from the sample are shown in table 3.3 and figure 3.2.

Table 3.3: Prevalence of bacteria species isolated from spoilt tomatoes

Bacterial isolates	Number of the isolates	Total bacterial isolates	% Prevalence
Vibrio spp.	9	46	19.56%
E.coli	8		17.39%
Klebsiella spp.	7		15.22%
Salmonella spp.	6		13.04%
Bacillus spp.	6		13.04%
Shigella spp.	5		10.87%
Staphylococcus spp.	3		6.52%
Enterobacter spp.	2		4.35%

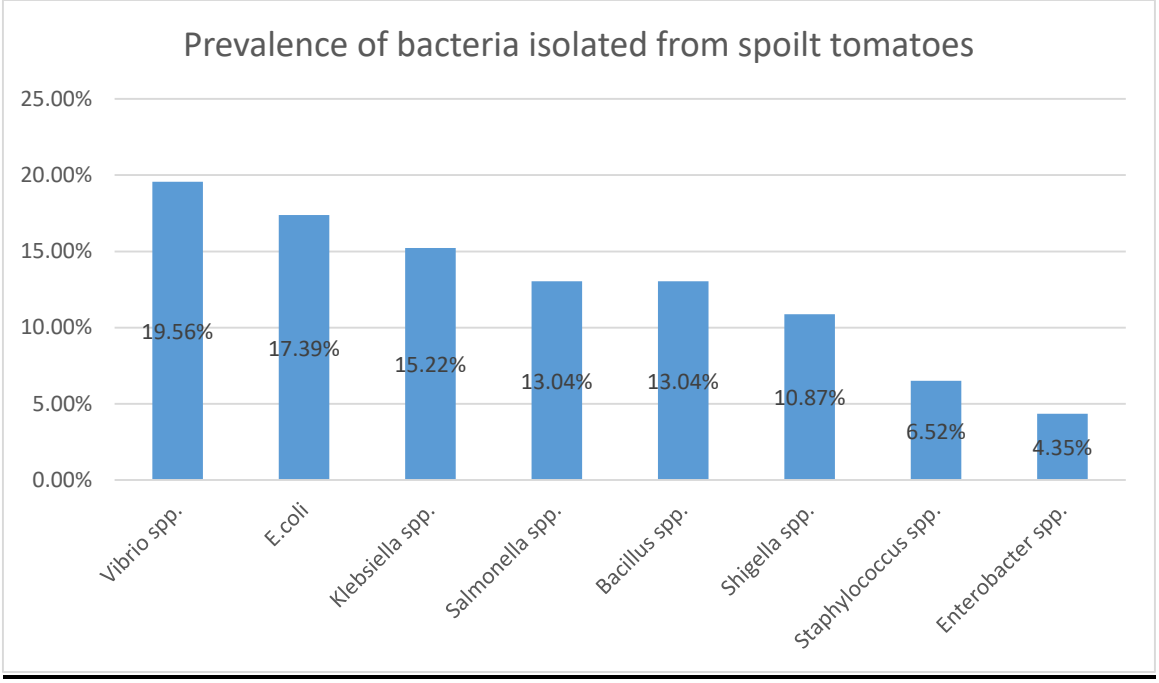


Figure 3.2 Prevalence of bacteria isolated from spoilt tomatoes

The identified isolates were both Gram negative and Gram positive organisms. Mostly, Gram negative bacteria were isolated including *E.coli*, *Klebsiella* spp, *Salmonella* spp, *Vibrio* spp, *Shigella* spp and *Enterobacter* spp. While, *Bacillus* spp and Staphylococcus spp were the only Gram positive bacteria identified.

Table 3.4: Classification of the isolates in comparison of Gram's Reaction

Gram's Reaction	**Number of isolates found**	**Percentage (%)**
Gram positive	9 (out of 46)	19.57
Gram negative	37 (out of 46)	80.43

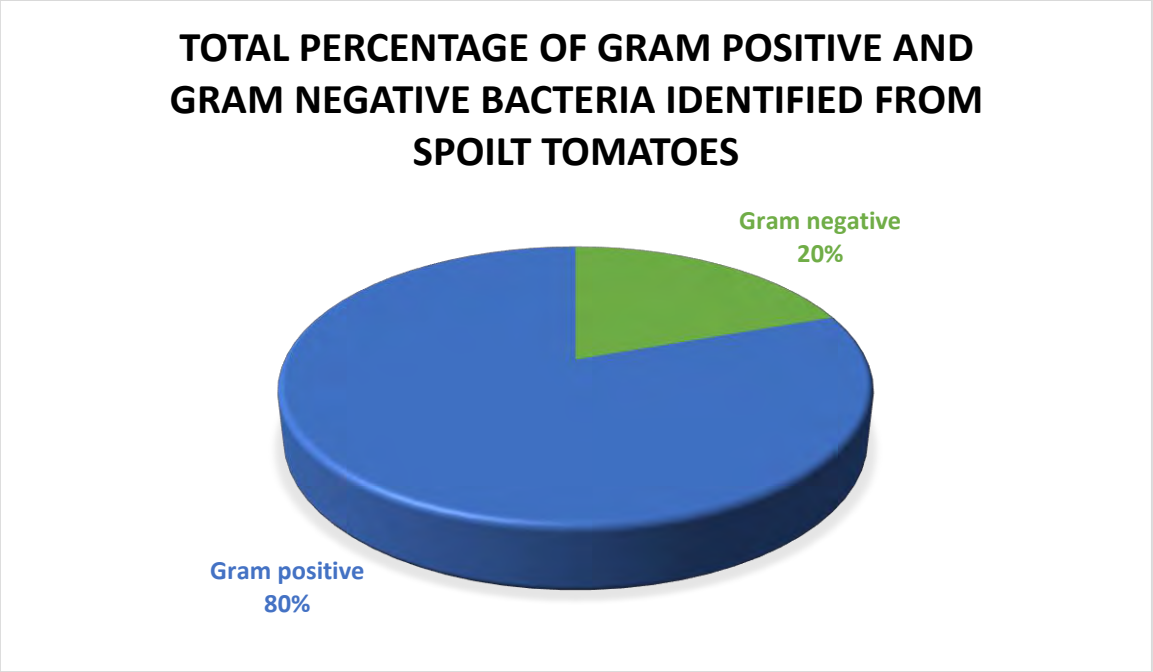


Figure 3.3: Total percentage of Gram positive and Gram negative bacteria identified from spoilt tomatoes

3.3 Temperature tolerance of the tested organism:

Temperature tolerance of the organisms was determined by growing the isolates in different temperatures like 4°C, 25°C and 60°C. All the isolates showed growth at 25°C but at 4°C and 60°C, no isolates showed viable growth.

Table 3.5: Temperature tolerance of bacterial isolates

Isolates ID	4°C	25°C	60°C
T1a (*E.coli*)	-	+	+
T1e (*Salmonella spp.*)	-	+	-
T1c (*Vibrio spp.*)	-	+	-
T1d (*Vibrio spp.*)	-	+	-
T1b (*Bacillus spp.*)	-	+	-
T1i (*E.coli*)	-	+	-
T1g (*Klebsiella spp.*)	-	+	-
T1h (*Enterobacter* spp.)	-	+	-
T1f (*Shigella spp.*)	-	+	-
T2b (*Bacillus spp.*)	-	+	-
T2d (*Salmonella spp.*)	-	+	-
T2g (*Staphylococcus spp.*)	-	+	-
T2f (*Klebsiella spp*)	-	+	-
T2c (*Vibrio* spp)	-	+	-
T2a (*E.coli*)	-	+	-
T2e (*Shigella spp*)	-	+	-
T3e (*Salmonella s*pp.)	-	+	-

Isolates ID	4°C	25°C	60°C
T3a (*E.coli*)	-	+	-
T3c (*Vibrio spp.*)	-	+	-
T3d *(Enterobacter spp.*)	-	+	-
T3f (*Shigella spp*)	-	+	-
T3h (*Staphylococcus spp*)	-	+	-
T3g (*Klebsiella* spp.)	-	+	-
T3b (*Bacillus spp*)	-	+	-
T4a (*E.coli*)	-	+	-
T4e (*Salmonella* spp.)	-	+	-
T4c (*Vibrio* spp.)	-	+	-
T4d (*Vibrio* spp.)	-	+	-
T4f (*Klebsiella* spp.)	-	+	-
T4b (*Bacillus spp*)	-	+	-
T5c (*Vibrio* spp.)	-	+	-
T5e (*Salmonella* spp.)	-	+	-
T5j (*Staphylococcus* spp.)	-	+	-
T5i (*E.coli*)	-	+	-
T5d (*Vibrio* spp.)	-	+	-
T5b (*Bacillus* spp.)	-	+	-
T5g (*Shigella* spp.)	-	+	-
T5h (*Klebsiella* spp.)	-	+	-
T5a (*E.coli*)	-	+	-
T6b (*Bacillus* spp.)	-	+	-
T6c (*Vibrio* spp.)	-	+	-
T6g (*Klebsiella* spp.)	-	+	-
T6a (*E.coli*)	-	+	-
T6d (*Salmonella* spp.)	-	+	-
T6e (*Shigella* spp.)	-	+	-
T6f (*Klebsiella* spp.)	-	+	-

(+) = growth (-) = no growth

Table 3.6: Total number of positive bacterial growth

Total bacterial isolates	**Bacterial growth at 4°C**	**Bacterial growth at 25°C**	**Bacterial growth at 60°C**
46	0(0%)	46 (100%)	0 (0%)

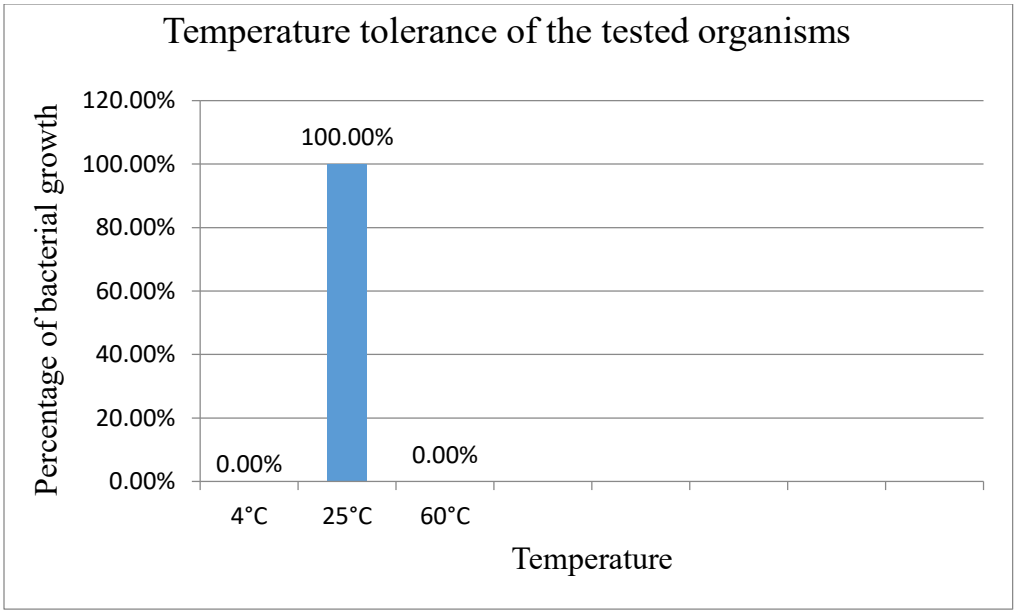


Figure 3.4: Temperature tolerance percentage of the tested organisms

3.4 Cellulase activity

Cellulase activity of abundant identified organisms is also observed

Table 3.7: Cellulose Hydrolysis test

Identified Organism	**Cellulose Hydrolysis**
Klesiella spp.	+
E.coli	-
Vibrio spp.	-
Shigella spp.	+
Bacillus spp.	-
Salmonella spp.	+
Shigella spp.	-

Chapter 4: Discussion and Conclusion

Discussion

Tomato is a fruit which has high percentage of water in it. This makes it more susceptible to various microorganisms. Losses of tomatoes due to spoilage is high. As tomatoes in Bangladesh are not generally carried in sterile condition rather it is brought to the Dhaka city in baskets and kept in exposed places of market where contamination from soil, air, and people can easily occur. Also, the water spread on the fruits and vegetables on local markets by the retailers to keep them fresh are not sterile, clean. As a result, these all increases the chances of contamination by microorganisms. Further, in developing countries like Bangladesh, India where people only cut off the spoilt part of fruits and vegetables rather than discarding the whole, the chances of contamination and the presence of organisms are high. As tomatoes are taken as raw in salads, the microorganisms present in spoiled tomatoes and the result of in taking contaminated or spoiled tomatoes should be known. (Abhinaba Ghosh, 2009)

Total of 46 isolates have been classified from the sample tomatoes brought from various markets of Dhaka city. These isolates were identified on basis of cultural and morphological characteristics of bacteria in various selective and differential media. In addition, traditional biochemical test results of these isolates were considered. Among the isolates, there were *Vibrio* spp with the highest number of 9 (19.56%), followed by *E.coli* with 8 (17.39%), *Klebsiella* spp of 7 (15.22%), *Salmonella* spp. of 6 (13.04%), *Bacillus* spp. of 6 (13.04%), *Shigella* spp of 5 (10.87%), Staphylococcus spp. of 3(6.52%) and *Enterobacter* spp. with least number of 2(4.65%).

Cellulose, and pectin are the polymeric compound present in tomatoes which help in spoilage of tomatoes. J. Raja Brindha et al., (2011). The presence of enzymes in microorganisms to degrade these polymeric compound enhance the spoilage of tomatoes. In this research, *Klebsiella* spp. *Shigella* spp. and *Salmonella* spp. were found to have the cellulase enzymes. While, the absence of cellulase enzymes were found in *E.coli*, *Vibrio* spp, and *Bacillus* spp. Further, the ability of these organisms to grow in different temperatures were tested. Since, these organisms were related directly or indirectly to the spoilage of tomatoes.

Growth of these identified organisms from the spoiled tomatoes in different temperatures were observed. The result emphasized storage of tomatoes in refrigerator rather than storing them in room temperature as all the microorganism could not grow in low temperature. Bacteria only gave positive growth in room tempeture. Furthermore, bacteria were not able to grow in high temperature (60^{o}C) indicating cooking would also help to kill the harmful microorganisms and to make the food healthy.

Conclusion:

The organisms found from the spoilt tomatoes in the current research, have the potential to cause various foodborne illness. Tomatoes have the possibility of becoming contaminated during various time of growing phase, post-harvesting, and transportation and at home or restaurant by cross contamination .Therefore, farmer to seller all should take precautionary measures to prevent contamination of tomatoes. Also, spoilt tomatoes should be discarded and not be consumed by humans.

References

References:

- Mahamud, F. , Dangora, D. , Mu'azu, S. , Khan, A. , Nura, S. and Gaiya, Z. (2013) Bio-chemical characterization of bacterial flora associated with spoilt vegetables in kaduna markets Northern Nigeria. *Advances in Biological Chemistry*, **3**, 141-145. doi: 10.4236/abc.2013.31017.

- J. Raja Brindha , T. Selva Mohan , G. Immanual , S. Jeeva and N. C. J. Packia Lekshmi (2011) Studies on amylase and cellulase enzyme activity of the fungal organisms causing spoilage in tomato. European Journal of Experimental Biology, 2011, 1 (3):90-96 https://pdfs.semanticscholar.org/6230/e7c78aea071cc26a8e8ffaeb67f9fa661bf8.pdf

- Puspanadan, S., 1 Afsah-Hejri, L., 1 Loo, Y.Y, 1 Nillian, E., 1 Kuan, C.H., 1 Goh, S.G., 1 Chang, W.S., 1 Lye, Y.L., 2 John, Y.H.T., 1 Rukayadi, Y., 3 Yoshitsugu, N.,3 Nishibuchi, M. and 1 Son, R. Detection of Klebsiella pneumoniae in raw vegetables using Most Probable Number-Polymerase Chain Reaction (MPN-PCR) International Food Research Journal 19(4): 1757-1762 (2012)

- Spyros D. Kominos, Charles E. Copeland, Barbara Grosiak, and Bosko Postic **(1972)**. Introduction of *Pseudomonas aeruginosa* into a Hospital via Vegetables
- Appl Microbiol. 1972 Oct; 24(4): 567–570.

- Alice S. Prince, in Principles and Practice of Pediatric Infectious Diseases (Fourth Edition), 2012 Etiologic Agents of Infectious Diseases.

- Masanori Toyofuku, Sang-Sun Yoon, in Advances in Microbial Physiology, Nitric Oxide and Other Small Signalling Molecules, 2018

- Jhalka Kadariya, Tara C. Smith, and Dipendra Thapaliya, "Staphylococcus aureus and Staphylococcal Food-Borne Disease: An Ongoing Challenge in Public Health," BioMed Research International, vol. 2014, Article ID 827965, 9 pages, 2014. https://doi.org/10.1155/2014/827965.

- Miedes, E., & Lorences, E. P. (2004). Apple (malus domestica) and tomato (lycopersicum) fruits cell-wall hemicelluloses and xyloglucan degradation during penicillium expansum infection.
  Journal of Agricultural and Food Chemistry, 52, 7957–7963.

- Margaret Barth, Thomas R. Hankinson, Hong Zhuang, and Frederick Breidt Microbiological Spoilage of Fruits and Vegetables

- V. H. Tournas. (2012) Spoilage of Vegetable Crops by Bacteria and Fungi and Related Health Hazards
  https://doi.org/10.1080/10408410590886024

- Ife Fitz James Bas Kuipers. Preservation of fruit and vegetables
  ISBN: 90 77073 302

- Onuorah Samuel. (2015) Fungi Associated with the Spoilage of Post-harvest Tomato Fruits Sold in Major Markets in Awka, Nigeria
  Universal Journal of Microbiology Research 3(2): 11-16, 2015
  DOI: 10.13189/ujmr.2015.030201

- Abhinaba Ghosh. (2009) IDENTIFICATION OF MICROORGANISMS RESPONSIBLE FOR SPOILAGE OF TOMATO (*LYCOPERSICON ESCULENTUM*) FRUIT. *Journal of Phytology 2009, 1(6): 414–416*

- Onuorah Samuel, Nriagu Ogonna, Obika Ifeanyi . (2016)
  Isolation, Characterization and Identification of Microorganisms from Spoilt Carrots Obtained from Ose Market Onitsha, Nigeria. Universal Journal of Biomedical Engineering 4(1): 6-9, 2016

- M.J. Uddin, C.K. Saha, M.M. Alam and M. Kabir. (2015). Post-Harvest losses of tomato at the fresh produce marketing chain in Bangladesh. Bangladesh J. Agri. Engg. 26 (1&2) 11-18 : 2015

- Bashir Omolaran Bello, habib Ullah, Odunayo Olawuyi J, Opeyemi Adebisi S, Alafe Azeez H, Owoade Temilade A. (2016) Microorganisms causing post-harvest tomato (Solanum lycopersium L.) fruit decay in Nergeria. 2016 ; 4(1): 374

- A.K.M. Amzad I-Iossain , M.L. Chadha , S.N. Mondal and S.M. Monowar Hossain. Techniques of Growing Tomato Under Summer Condition http://203.64.245.61/fulltext_pdf/EAM/1991-2000/eam0164.pdf

- M. Celia Marín-Rodríguez, John Orchard, Graham B. Seymour; Pectate lyases, cell wall degradation and fruit softening, *Journal of Experimental Botany*, Volume 53, Issue 377, 1 October 2002, Pages 2115–2119, https://doi.org/10.1093/jxb/erf089

Appendices

Appendix-I

Media compositions

The compositions of all media used in the study are given below:

Nutrient agar

Component	Amount(g/l)
Peptone	5.0
Sodium chloride	5.0
Beef extract	3.0
Agar	15.0
Final pH	7

Saline

Component	Amount(g/l)
Sodium chloride	9.0

Mannitol salt agar

Component	Amount(g/l)
Proteose peptone	10.0
Beef extract	1.0
Sodium chloride	75.0
D-mannitol	10.0
Phenol red	0.025
Agar	15.0
pH	7.4 ±0.2 at 25^0 C

Eosine methylene Blue (EMB) Agar

Component	Amount(g/l)
Peptone	10.0
Dipotassium phosphate	2.0
Lactose	5.0
Sucrose	5.0
Eosin yellow	0.14
Methylene blue	0.065
Agar	13.50
pH	7.1 ±0.2 at 25^0 C

Bacillus cereus (BC) agar

Component	Amount(g/l)
Peptic digest of animal tissue	1.0
Mannitol	10.0
Sodium chloride	2.0
Magnesium sulphate	0.1
Dipotassium phosphate	2.5
Monopotassium phosphate	0.25
Sodium pyruvate	10.0
Bromo thymol blue	0.12
Agar	15.0
pH	7.1 ±0.2 at 25^0 C

Salmonella Shigella agar

Component	Amount(g/l)
Peptic digest of animal tissue	15.0
Proteose peptose	5.0
Dextrose	1.0
Lead acetate	0.2
Sodium thiosulphate	0.08
Agar	15.0
pH	7.0 ±0.2 at 25^0 C

TCBS agar

Component	Amount(g/l)
Proteose peptose	10.0
Yeast extract	5.0
Sodium thiosulphate	10.0
Sodium citrate	10.0
Oxgall	8.0
Sucrose	20.0
Sodium chloride	10.0
Ferric citrate	1.0
Bromo thymol blue	0.04
Thymol blue	0.04
Agar	15.0
pH	8.6 ±0.2 at 25^0 C

Cetrimide agar

Component	Amount(g/l)
Agar	15.0
pH	7.0 ±0.2 at 25^0 C

Simmon's Citrate agar

Component	Amount(g/l)
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Bacto agar	15.0
Bacto bromo thymol blue	0.08

Methyl Red-Vogues Proskauer (MR-VP) media

Component	Amount(g/l)
Peptone	7.0
Dextrose	5.0
Dipotassium hydrogen phosphate	5.0
pH	7.0

Triple Sugar Iron (TSI) agar

Component	Amount(g/l)
Bio-polytone	20.0
Sodium chloride	5.0
Lactose	10.0
Sucrose	10.0

Dextrose	1.0
Ferrous ammonium sulphate	0.2
Sodium thiosulphate	0.2
Phenol red	0.0125
Agar	13.0
pH	7.3

Motility Indole Urease (MIU) agar

Component	**Amount(g/l)**
Tryptone	10.0
Phenol red	0.1
Agar	2.0
Sodium chloride	5.0
pH	6.8 ±0.2 at 25^0 C

Starch agar

Component	**Amount(g/l)**
Meat extract	3.0
Peptic digest of animal tissue	5.0
Starch (soluble)	2.0
Agar	15.0
pH	7.2 ±0.2 at 25^0 C

Skim Milk agar

Component	**Amount(g/l)**
Skim milk agar	28.0
Casein enzymic hydrolysate	5.0
Yeast extract	2.5
Dextrose	1.0
Agar	15.0
pH	7.0 ±0.2 at 25^0 C

Congo red agar

Component	**Amount(g/l)**
CMC powder	2.0
$NaNO_3$ / KNO_3	1.0
K_2HPO_4	1.0
KCL	0.5
$FeSO_4$	0.01
Yeast extract	5.0
Agar	15.0
pH	7.2 ±0.2 at 25^0 C

Appendix-II

Reagents and buffers

Gram's iodine (300ml)

To 300ml distilled water, 1gram iodine and 2 gram potassium iodide was added. The solution was mixed on a magnetic stirrer overnight and transferred to a reagent bottle and stored at room temperature.

Crystal Violet (100ml)

To 29ml 95% ethyl alcohol, 2gm crystal violet was dissolved. To 80ml distilled water, 0.8gm ammonium oxalate was dissolved. The two solutions were mixed to the stain and stored in a reagent bottle at room temperature.

Safranin (100ml)

To 10ml 95% ethanol, 2.5gm safranin was dissolved. Distilled water was added to the solution to make a final volume of 100ml. The final solution was stored in a reagent bottle and stored in room temperature.

Kovac's reagent (150ml)

To 150ml (reagent grade) isoamyl alcohol, 10gm of p-dimethylaminobenzaldehyde (DMAB) and 50ml of HCL (concentrated) were added and mixed. Next, the prepared reagent was kept in an aluminum foiled reagent bottle to prevent light exposure and stored at 4^0 C.

Methyl Red (200ml)

To 1gm of methyl red powder, 300ml of 95% ethanol was completely dissolved. Next, 200ml distilled water was added to make 500ml of 0.05 %(wt/vol) solution in 60 %(vol/vol) ethanol and stored at 4^0 C.

Barrit's Reagent A (100ml)

5% (wt/vol) α-naphthol was added to 100ml absolute ethanol and stored at 4^0 C.

Barrit's Reagent B (100ml)

40% (wt/vol) KOH was added to 100ml absolute ethanol and stored at 4^0 C.

Catalase reagent (20ml 3% hydrogen peroxide)

From a stock solution of 35% hydrogen peroxide, 583µl solution was added to 19.417ml distilled water and stored at 4^0 C.

Urease reagent (50ml 40% urea solution)

To 50ml distilled water, 20gm pure urea powder was added. The solution was filtered through a syringe filter and collected into a falcon tube and stored at 4^0 C.

Appendix-III

Instruments

Autoclave	Model: WIS 20R Daihan Scientific Co.ltd, Korea
Laminar airflow cabinet	Model-SLF-V, vertical, SAARC group Bangladesh
Incubator	Model-OSI-500D, Digi system Laboratory Instruments Inc. Taiwan
Vortex mixer	Digi system Taiwan, VM- 2000
Electronic balance	Model: WTB 200 RADWAG Wagi Electronics
Refrigerator (4^0 C)	Model: 0636 Samsung